TABLE 1

F344 Rat ♂ 4~8 weeks old

⇩

Isolation of Hepatic Cells (Collagenase Perfusion)

⇩

Percoll Centrifugation

⇩

Hepatocytes

6×10^5 cells / 3.5cm dish (without collagen coat)

37 ℃, 5% CO_2, 95% Air

DMEM, 44mM $NaHCO_3$, 20mM HEPES, 0.5mg/l Insulin

10^{-7}M Dexamethasone, 30mg/l L-prolin

penicillin and streptmycin

⇩ 2~3 hours

Medium Change

DMEM, 10% FBS, 44mM $NaHCO_3$, 20mM HEPES

0.5mg/l Insulin, 10^{-7}M Dexamethasone

10mM Nicotinamide, 10ng/ml EGF

0.2mM L-ascorbic acid phosphate

penicillin and streptmycin

⇩ 4 days

Pre-confluent

Subculture with 0.02% EDTA

1% DMSO

The same operations were carried out through systems added with additive factors such as FCS, nicotinamide, EGF, L-ascorbic acid-phosphate except for one, to investigate the effects of the individual additives on the hepatocytes and the non-parenchymal cells.

As a result of the above, a treatment with 0.02% EDTA caused the hepatocytes to detache them in the form of clusters, and as shown in Fig. 1 (first subculture × 29.4), the clusters adhered to the dish within one or more days of subculture (Fig. 1(a): the second day of subculture), grew from the third day or so of subculture (Fig. 1 (b): the fifth day of subculture), and part of cells died and peeled off on seventh day or so of subculture (Fig. 1 (c): the eighth day of subculture).

About the eighth day or so of subculture and thereafter, the surviving hepatocytes grew (Fig. 1 (d): the 44th day of subculture), and in the case of most proliferative clusters, the number of hepatocytes increased 5-fold on the 41th day of subculture (Fig. 2).

Growth of the hepatocytes after subculture was confirmed from the increase in number of the hepatocyte clusters, incorporation of BrdU, and mitotic figure. Incorporation of BrdU was observed in many hepatocytes on the 30th day of subculture (Fig. 3 (a)). Figs. 3 (a), (b) and (c) show the 30th, the 45th and the 50th days of the first generation of subculture, respectively.

In Fig. 3(a), double staining (× 100) was applied with BrdU (brown)-transferrin (red); Fig. 3(b) is based on stain (× 606) with α_1-antitrypsin (brown); and in Fig. 3(c), staining (× 606) was applied with albumin (brown). In the hepatocytes continuing to grow after subculture, expression of albumin, α_1-antitrypsin, and transferrin